

# Virulence Determination for Rapid Extraintestinal Dissemination (Acute Infection) of Common *Salmonella* Serotypes in Swine

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**Abstract:** *Salmonella enterica* (Typhimurium and Choleraesuis) have been shown to rapidly disseminate extraintestinally (RED) within 3 hours of intranasal inoculation in pigs (Fedorka-Cray et al., 1995) (Blaha et al., 1997) (Nugent et al., 2000) (Nielsen, 2000). Evaluation of RED serotypes may be an important indicator of *Salmonella* virulence. Experimentally, pigs were challenged with important lymph node, fecal and vaccine isolates of *Salmonella* and evaluated for RED. These isolates include: *Salmonella* Heidelberg, *Salmonella* Infantis, *Salmonella* Derby, *Salmonella* Worthington, *Salmonella* 4, 12 i-monophasic, *Salmonella* untypable HL 10416, *Salmonella* Typhimurium, *Salmonella* Typhimurium variant Copenhagen, *Salmonella* Bredeney, *Salmonella* Muenchen, *Salmonella* Brandenburg, *Salmonella* Choleraesuis SC-38, *Salmonella* Choleraesuis SC-54, and *Salmonella* Choleraesuis strain Argus. Three hours after intranasal inoculation, the pigs were euthanized, necropsied, and the following tissues were collected for qualitative isolation: tonsil, thymus, blood, mandibular lymph node, lung, spleen, liver, ileocecal lymph node, colon contents and cecum contents. Fewer tissues were positive for vaccine strains as compared to wild type or parent strains.

**Keywords:** RED, pathogenesis, intranasal

**Introduction:** It has been shown that pigs in transit to slaughter and while in lairage have contracted infections of *Salmonella* which were likely not present on the farm of origin prior to transfer (Hurd et al., 2001). It is implied by Hurd et al. that these animals may have encountered these new and different species of *Salmonella* from the short term intermingling of pigs in transit and while in lairage. This evidence supports previous findings, in which an unknown alternate route of invasion by *Salmonella* Typhimurium was suspected, after *Salmonella* was found in body organs and lower intestine within 3 hours after intranasal inoculation of

esophagostomized pigs (Fedorka-Cray *et al.*, 1995). The objectives of this study were to determine if wild type lymph node isolates, fecal isolates, and vaccine strains are capable of rapid extraintestinal dissemination in the pig.

**Materials and Methods:** Pigs were obtained from herds known to have low levels of *Salmonella* at 3-4 weeks of age. Five separate controlled trials were conducted in which various wild type serotypes and vaccine strains were inoculated into 4 pigs each. Uninoculated control pigs were included in each trial.

Upon arrival to Iowa State University the pigs were acclimatized to the facility for 4-8 days. During acclimatization, blood and feces from the pigs were evaluated for *Salmonella* status by the Danish Mix-ELISA and culture. Inoculum was prepared as described (Fedorka-Cray *et al.*, 1995). Pigs were euthanized with pentobarbital sodium at 3 hours post-inoculation and necropsied. At necropsy 3-5 gram samples of tonsil, thymus, mandibular lymph node, lung, spleen, liver, ileocecal lymph node, and colon contents were collected for isolation of *Salmonella*. Additionally, 25 grams of cecum contents and 25 mls of blood were collected for *Salmonella* isolation.

For isolation of *Salmonella*, samples were hammered with a plastic headed mallet until well minced. Samples were then diluted in Buffered Peptone Water (BPW) 1:9, hand mixed, and incubated at 37° C for 18 hours. After incubation in BPW the samples were vortexed and 100µl was transferred from the BPW to 9.9ml tubes of rappaport-vassiliadis (RV) broth, and incubated at 42° C overnight. From RV, samples were streaked for isolation on XLD agar plates, and incubated overnight at 37° C. Selected colonies from each tissue were verified as *Salmonella* by biochemical and serologic tests.



**Results and Discussion:** The current practice of intermixing swine of different origins during transport and lairage has resulted in the opportunity for *Salmonella* in infected herds to contaminate pigs of uninfected herds within three hours of co-mingling (Hurd, 2001). This study evaluated the ability of wild type isolates and two vaccine strains for their abilities to rapidly disseminate extraintestinally (Table 1). Since all wild type isolates appeared to disseminate rapidly and caused acute infection, future intervention strategies will need to address this aspect of the common human foodborne *Salmonella*. Percentage of organs positive for

**Table 1. Percentage of organs positive for *Salmonella* isolation.**

\* *Salmonella* were isolated from three tissues in two of six negative control pigs. .

Serotype	Percent of Organs positive
S. Heidelberg	83%
S. Worthington	68%
S. 4, 12 i-monophasic	60%
S. Bredeney	58%
S. Infantis	56%
S. Derby	55%
S. Untypable	53%
S. Muenchen	53%
S. Brandenburg	48%
S. Typhimurium variant Copenhagen	45%
S. Typhimurium	41%
S. Choleraesuis Strain 38	40%
S. Choleraesuis Strain Argus	20%
S. Choleraesuis Strain 54	10%
Controls	5%*

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